

AMENDMENTS TO THE CLAIMS

Listing of Claims

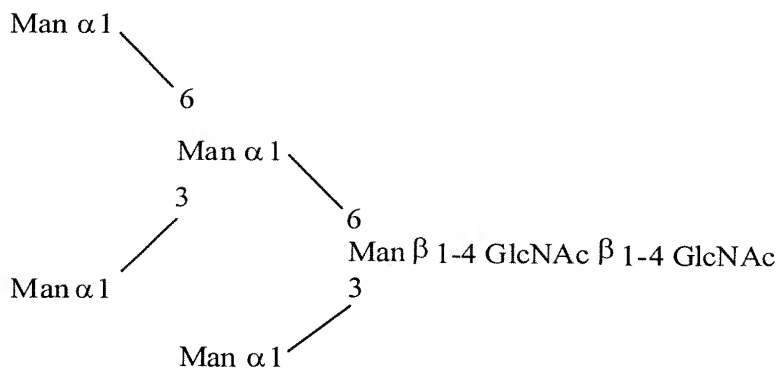
1. (Previously Presented) A process for producing a methylotrophic yeast that produces a mammalian type sugar chain, which comprises the steps of:

- 1) disrupting an *OCH1* gene which encodes α -1,6-mannosyl transferase and YPS1 gene which encodes Aspartic protease 3, in a methylotrophic yeast; and
- 2) introducing an α -1,2-mannosidase gene into the yeast and expressing it therein,

wherein the methylotrophic yeast belongs to the genus *Pichia* or *Ogataea*.

2. (Previously Presented) A process according to claim 1, wherein the mammalian type sugar chain is represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



3. (Canceled)

4. (Original) A process according to claim 1 or 2, wherein the methylotrophic yeast is *Ogataea minuta*.

5. (Original) A process according to claim 1, wherein the methylotrophic yeast is a strain from *Ogataea minuta* strain IFO 10746.
6. (Original) A process according to claim 1, wherein the α -1,2-mannosidase gene is expressed under the control of a methanol-inducible promoter.
7. (Original) A process according to claim 6, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.
8. (Original) A process according to claim 7, wherein the alcohol oxidase (*AOX*) gene is from *Ogataea minuta*.
9. (Previously Presented) A process according to claim 1, characterized in that the α -1,2-mannosidase expressed from the α -1,2-mannosidase gene further comprises a yeast endoplasmic reticulum (ER) retention signal.
10. (Original) A process according to claim 1, wherein the α -1,2-mannosidase gene is from *Aspergillus saitoi*.
11. (Original) A process according to claim 1, which further comprises a step of transforming a heterologous gene into the yeast.
12. (Original) A process according to claim 11, wherein the heterologous gene is transferred using an expression vector and is expressed in the yeast.
13. (Original) A process according to claim 12, wherein the expression vector comprises a methanol-inducible promoter.
14. (Original) A process according to claim 13, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.

15. (Original) A process according to claim 14, wherein the alcohol oxidase (AOX) gene is from *Ogataea minuta*.

16. (Original) A process according to claim 12, wherein the expression vector comprises a promoter of a glyceraldehyde-3 -phosphate dehydrogenase (*GAPDH*) gene.

17. (Original) A process according to any one of claims 11 to 16, wherein 20% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.

18. (Original) A process according to any one of claims 11 to 16, wherein 40% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.

19. (Original) A process according to any one of claims 11 to 16, wherein 60% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.

20. (Original) A process according to any one of claims 11 to 16, wherein 80% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.

21. (Original) A process according to any one of claims 11 to 16, wherein the protein encoded by the heterologous gene is from humans.

22. (Original) A process according to any one of claims 11 to 16, wherein the protein encoded by the heterologous gene is an antibody or a fragment thereof.

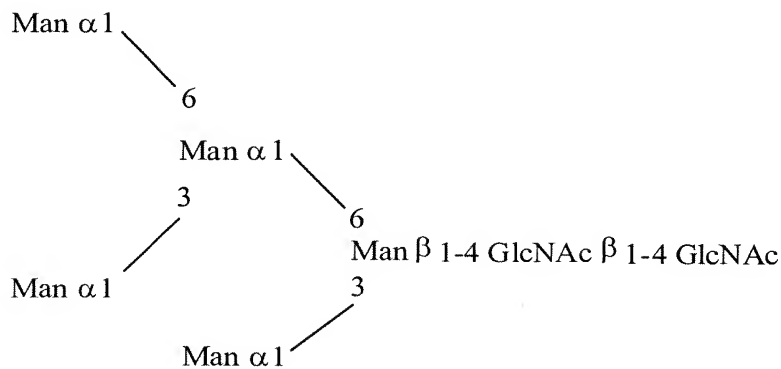
23. (Currently Amended) A methylotrophic yeast produced by a process according to claim 1 or claim 11.

24. (Currently Amended) A process for producing a protein encoded by a heterologous gene, wherein the process comprises culturing the methylotrophic yeast produced by a process according to claim 11 of claim 23 in a medium to obtain the protein encoded by the heterologous gene comprising a mammalian type sugar chain from the culture.

25.–93. (Canceled)

94. (Previously Presented) A process for producing an *Ogataea minuta* strain, which produces a mammalian type sugar chain represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



comprising a step of disrupting *OCH1* gene in the *Ogataea minuta* strain; and a step of disrupting a *YPS1* gene in the same strain.

95. (Original) A process of claim 94, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

96. (Previously Presented) A process according to claim 94, which further comprises a step of disrupting at least one gene selected from the group consisting of a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15, an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27, an *HIS3* gene comprising

the nucleotide sequence represented by SEQ ID NO:99, and a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

97. (Previously Presented) A process according to claim 94, which further comprises a step of disrupting at least one gene selected from the group consisting of a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51, a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57.

98. (Original) A process according to claim 97, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63 and/or an *MNN9* gene comprising the sequence represented by SEQ ID NO:69.

99. (Original) A process according to any one of claims 94 to 98, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene from *Aspergillus saitoi*.

100. (Original) A process according to claim 99, wherein the α -1,2-mannosidase gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the α -1,2-mannosidase gene; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

101. (Original) A process according to claim 94, which further comprises a step of introducing and expressing a *PDI* gene.

102. (Previously Presented) A process according to claim 101, wherein the *PDI* gene is a gene from *Saccharomyces cerevisiae* with the sequence found at GenBank Accession number M62815.

103. (Original) A process according to claim 102, wherein the *PDI* gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the *PDI* gene; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

104. (Original) A process according claim 94, which further comprises a step of introducing and expressing a heterologous gene.

105. (Original) A process according to claim 104, wherein the heterologous gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the heterologous gene; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

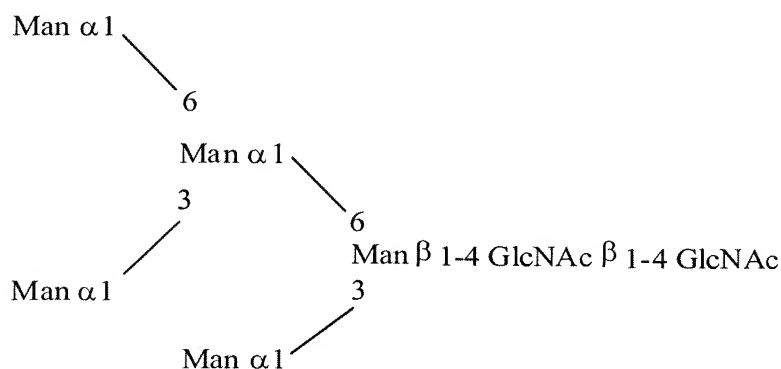
106. (Original) A process for producing a protein encoded by a heterologous gene, which comprises culturing *Ogataea minuta* produced by the process of claim 104 in a

medium, to obtain the protein comprising a mammalian type sugar chain encoded by the heterologous gene from the culture.

107. (Canceled)

108. (Previously Presented) A process for producing an *Ogataea minuta* strain, which produces a mammalian type sugar chain represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



wherein the process comprises the steps of:

disrupting an *OCH1* gene comprising the nucleotide sequence represented by SEQ ID NO:42 in an *Ogataea minuta* strain; and

disrupting a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15 in the same strain; and

disrupting a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51 in the same strain; and

disrupting a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57 in the same strain; and

disrupting a *YPS1* gene comprising the nucleotide sequence represented by SEQ ID NO:115 in the same strain.

109. (Original) A process according to claim 108, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

110. (Original) A process according to claim 108 or 109, which further comprises a step of disrupting an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27.

111. (Original) A process according to claim 110, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63.

112. (Original) A process according to claim 111, which further comprises a step of disrupting an *HIS3* gene comprising the nucleotide sequence represented by SEQ ID NO:99.

113. (Original) A process according to claim 111, which further comprises a step of disrupting a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

114. (Canceled)

115. (Previously Presented) A process according claim 108, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene.

116. (Previously Presented) A process according to claim 115, wherein the α -1,2-mannosidase gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the α -1,2-mannosidase gene; and

- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

117. (Previously Presented) A process according to claim 108, which further comprises a step of introducing and expressing a *PDI* gene from *Saccharomyces cerevisiae* with the sequence found at GenBank Accession number (M62815).

118. (Previously Presented) A process according to claim 117, wherein the *PDI* gene (M62815) is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the *PDI* gene with the sequence found at GenBank Accession number M62815; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

119. (Previously Presented) A process according to claim 108, which further comprises a step of introducing and expressing a heterologous gene.

120. (Previously Presented) A process according to claim 119, wherein the heterologous gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the heterologous gene; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

121. (Previously Presented) A process for producing a protein encoded by a heterologous gene comprising a mammalian type sugar chain, wherein the process comprises culturing *Ogataea minuta* produced by the process of claim 119 in a medium to obtain the protein from the culture.

122. (Canceled)

123. (Previously Presented) The process of claim 9, wherein the yeast endoplasmic reticulum (ER) retention signal has the sequence of SEQ ID NO: 121.

124. (Previously Presented) The process of claim 94, wherein the *OCH1* gene has the sequence of SEQ ID NO: 42.

125. (Previously Presented) The process of claim 94, wherein the YPS1 gene has the sequence of SEQ ID NO: 115.